NAME:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

IB Biology Lab Investigation

*AIM: Experimental investigation of a factor affecting enzyme activity. (Practical 3)*

**Part 1: Effect of Temperature on Enzyme Function**

Bromelin is an enzyme used in meat tenderizer. It is one of a family of plant enzymes, including enzymes from pineapple and fig, which break down proteins. This is why the directions on a box of Jello remind you never to use fresh or frozen pineapple in your gelatin, since gelatin is the protein responsible for the “gel”.

In this experiment you will observe the effect of bromelin on gelatin. You will also test the effect of temperature (boiling or chilling) on the function of the bromelin.

Procedure

1. Prepare a gelatin solution by heating 6.0 g of gelatin in 100 ml water until dissolved. Gently mix, do not boil. Cool to room temperature.

2. Label test tubes 1-5

3. Add reactants as follows:

Tube 1: 5ml Gelatin Solution + 5ml Water

Tube 2: 5ml Gelatin Solution + 5ml Bromelin Solution

Tube 3: 5ml Gelatin Solution + 5ml Chilled Bromelin Solution

Tube 4: 5ml Gelatin Solution + 5ml Boiled Bromelin Solution

Tube 5: 5ml Gelatin Solution + 5ml Water + meat tenderizer

4. Mix tubes

7. Place tubes in ice water for 10 mins.

8. PREDICT what you think will occur in each tube.

8. Observe tubes every 5 mins by removing from ice bath and noting the degree of gelatinization.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Tube | Prediction | Observation @10 min | Observation@ 15min | Observation@20 min |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |
| 4 |  |  |  |  |
| 5 |  |  |  |  |

**Part 2: Effect of Temperature on Enzyme activity**

For this part of this experiment you will be testing for the digestion of carbohydrates by enzymes. To complete this test you will use Iodine to test for starch. Iodine turns blue/black in the presence of starch but not simple sugars.

To make the starch solution: Crush and grind one saltine cracker into a fine powder with mortar & pestle. Add ground cracker to a 50ml beaker and add 30ml of water and mix well. Pour the mixture into a different 50ml beaker using a section of cloth to strain the solids from the solution. Label the liquid Starch Solution. Wash all glassware with soapy water and dry.

To make the Enzyme Solution: Carefully open one capsule and empty the contents into a clean mortar. Crush and grind the contents, while slowly adding 10ml of water. Once the contents have be completely crushed and mixed, use a clean pipette to transfer the enzyme solution to a conical tube. You may have to rinse several times to get all the solution.

Once you have made your solutions you are ready to begin the experiment.

1. Label test tubes 1-6

2. Add reactants as follows (use a different pipette for each reactant)

Tube 1: 0.5ml Starch Solution + 0.5ml Water @ 20°C for 10 mins

Tube 2: 0.5ml Starch Solution + 0.5ml Enzyme Solution @ 20°C for 10 mins

Tube 3: 0.5ml Water + 0.5ml Enzyme Solution @ 20°C for 10 mins

Tube 4: 0.5ml Starch Solution + 0.5ml Water, Incubated @ 37° for 10 mins

Tube 5: 0.5ml Starch Solution + 0.5ml Enzyme Solution, Incubated @ 37° for 10 mins

Tube 6: 0.5ml Water + 0.5ml Enzyme Solution, Incubated @ 37° for 10 mins

3. Place tubes 1,2,3 at room temp for 10 minutes.

4. Place Tubes 4,5,6 in water bath. Monitor temp and maintain at 37° for 10 mins

5. PREDICT what you think the result will be for each tube.

6. Add 1 drop Iodine to each tubes and record results (color). Place in test tube rack. Wait 10 mins and check again. Record results (color).

|  |  |  |  |
| --- | --- | --- | --- |
| Tube | Prediction | Results (10min incubation) | Results after additional 10m |
| 1 |  |  |  |
| 2 |  |  |  |
| 3 |  |  |  |
| 4 |  |  |  |
| 5 |  |  |  |
| 6 |  |  |  |

**Part 3: Effect of pH on protein digestion**

For this part of the lab you will be observing the effect of pH on the activity of our enzyme solution. Depending on where an enzyme works in the digestive system, some work best in acidic environments while others work best in neutral pH. Additionally at certain pH enzymes may denature. In this test you will expose protein to enzymes solution at various pH and compare the relative amount of digestion.

To make the Enzyme Solution: Carefully open one capsule and empty the contents into a clean mortar. Crush and grind the contents, while slowly adding 10ml of water. Once the contents have be completely crushed and mixed, use a clean pipette to transfer the enzyme solution to a conical tube. You may have to rinse several times to get all the solution.

1. Obtain boiled egg white and cut into equal size cubes approximately .5cm3

2. Label test tubes 1-6

3. Add reactants as follows:

Tube 1: Egg white + 2ml water + 3 drops water

Tube 2: Egg white + 2ml enzyme solution + 3 drops water

Tube 3: Egg white + 2ml enzyme solution + 3 drops weak acid (acetic acid)

Tube 4: Egg white + 2ml enzyme solution + 3 drops weak base (bicarbonate)

Tube 5: Egg white + 2ml enzyme solution + 3 drops strong acid (citric acid)

Tube 6: Egg white + 2ml enzyme solution + 3 drops strong base (NaOH)

4. Place tubes in a water bath maintained at 37°C

5. PREDICT what you think will occur in each tube

5. OBSERVE tubes at time zero, 10min, 20min. Record observations in table below;

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| tube | prediction | Time 0 | Time 10min | Time 20min |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |
| 4 |  |  |  |  |
| 5 |  |  |  |  |
| 6 |  |  |  |  |

**Part 4: Effect of emulsifier on lipid digestion**

For this part of the experiment you will be examining the effect of bile (an emulsifier) on the digestion of fats by the enzyme solution

Lipids are insoluble in water. This poses a problem for digestion, as their separation in water leaves little surface area for digestive enzymes to access. For lipid digestion to begin, the large lipid droplet must be broken into many smaller droplets. This process is called *emulsification* and the resulting solution is termed an emulsion. In the digestive system, bile is produced by the liver and stored in the gall bladder to emulsify lipids in the small intestine, where pancreatic lipase is released. In order for the lipase to break down the fat into fatty acids, the lipase must be able to attack the molecules. Once lipase breaks down fats we see a change in the pH which we can test using a *titration*. Phenolphthalein is a substance which turns bright pink at pH 10. We will slowly add NaOH to the solution until we observe the pink color. The more base needed to reach the pink color, the lower the initial pH (more acid).

To make the Enzyme Solution: Carefully open one capsule and empty the contents into a clean mortar. Crush and grind the contents, while slowly adding 10ml of water. Once the contents have be completely crushed and mixed, use a clean pipette to transfer the enzyme solution to a conical tube. You may have to rinse several times to get all the solution.

1) Label tubes 1-6

2) Add reactants as follows:

Tube 1,2: 1ml Oil + 2ml water

Tube 3,4: 1ml Oil + 1ml Enzyme Solution + 1ml water

Tube 5,6: 1ml Oil + 1ml Enzyme Solution + 1 ml Emulsifier

3) Place in a water bath and maintain for 5 mins (Tubes 1,3,5) and for 20 mins (2,4,6).

4) PREDICT what you think will occur in each tube

5) After the time has elapsed remove tubes from water bath.

6) Swirl the tubes and OBSERVE – Record Observations

7) Add 2 drops of phenolphthalein to Tube 1

8) SLOWLY add one drop NaOH the test tube, gently swirling the tube after each drop. Continue adding one drop at a time of COUNTING the number of drops, until a bright pink color is achieved and remains after gentle swirling.

9) Repeat steps 5-9 with all test tubes.

|  |  |  |  |
| --- | --- | --- | --- |
| Tube | prediction | observations | Number of drops NaOH |
| 1 |  |  |  |
| 2 |  |  |  |
| 3 |  |  |  |
| 4 |  |  |  |
| 5 |  |  |  |
| 6 |  |  |  |